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Homology modelling and simulation of *Wolbachia* surface protein (WSP) of Uzifly and study of its *Insilico* protein-protein anti-apoptosis interaction process with ethanol stressed HepG2 Cell line pathway proteins

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Abstract
Aim: The main objective of this study was to investigate the pharmacological effect of modelled *Wolbachia* Surface Protein (WSP) on Ethanol stressed HepG2 cell line and the role of WSP in Anti-apoptosis process.
Materials and Methods: The sequence of WSP protein was retrieved from protein databases and subjected to homology modelling. The modelled structure was validated using online tools and softwares and energy minimization was introduced to improve the total quality of the structure. The fine refined structure model of WSP was docked with various proteins involved in Ethanol stressed HepG2 cell line. Finally, the best scored protein-protein interaction was reported.
Results and Discussion: During homology modelling with various templates, the Protein Data Bank (PDB) ID: 1P4T_A a Neisserial Surface Protein A showed good sequence alignment, least positives with the best Discrete Optimized Protein Energy (DOPE) score of -11,757.60 and 0.611Å root-mean-square deviation (RMSD). Further docking of WSP with seven crucial proteins involved in Ethanol stressed HepG2 cell line was done. Among the seven crucial proteins, apoptosis inhibition was more Favoured through the Fas ligand (Fas L) and c-Jun N-terminal kinases with best energy score of value -34.0318 kcal/mol and -33.16 kcal/mol respectively.
Conclusion: Hence, the WSP interaction was studied only with the top scored complex of Fas ligand (Fas L). From the results, it was clearly revealed that anti-apoptosis in Ethanol stressed HepG2 cell line is more Favoured through Fas L and thereby it protects the HepG2 cells from damage. Thus WSP might have a significant therapeutic effect on Ethanol-related liver diseases.

Keywords: Anti-apoptosis, c-Jun n-terminal kinases, Fas ligand, homology modelling, hepg2 cell line, *Wolbachia* surface protein (WSP)

Introduction
In the worldwide, Chronic liver diseases (CLDs) is a major public health issue. According to recent statistics in 2017, 844 million people were affected by Chronic liver diseases, with a mortality rate of 2 million per year [1]. The prevalence and incidence of CLDs are high in Western countries because of poor health practice and high alcohol consumption. Also, it is found to be a major health problem than compared with that of other chronic diseases like cardiovascular, diabetes and pulmonary [1-2]. The main cause of this problem is alcohol consumption that leads to hepatocyte apoptosis and inflammation, further significant excessive consumption of alcohol may damage the liver cells and its role [3]. Hence, it is necessary to find the best cost-effective treatment for the alcohol related liver diseases and to create more public awareness among people to prevent the mortality.
HepG2 cell lines are derived from tumor liver tissue [4]. HepG2 has been widely used for the identification of cytotoxic, genotoxic and in addition cytoprotective, antigenotoxic and cogenotoxic materials [5]. While, growing at in-vitro conditions these cells secrets various plasma proteins such as albumin, plasminogen, fibrinogen etc. [6].
HepG2 cell line with ethanol stress are more favourable to study due to the modification of both anti-apoptotic and pro-apoptotic mechanisms in the ethanol induced apoptosis in HepG2 cells. It has also been stated that FAS receptor activation in low ethanol-induced apoptosis in human hepatocellular carcinoma (HepG2) cells activates the caspase-8, intracellular adapter protein FADD (Fas-Associated Death Domain) and mitogen-activated protein kinase (MAPK) pathway [7].
The primary objective of this study was to investigate the anti-apoptosis binding process.

Therefore, in order to study its protein-protein interaction, bioinformatics application was introduced. Initially, the list of proteins involved in the apoptosis pathways was chosen after screening process which was carried out based on the literature survey. Finally, only seven therapeutically significant drug target proteins, that majorly involved in the programmed cell death pathway was chosen and docked with another small molecular weight protein WSP (of *Wolbachia* endosymbiont).

Thus, the sequence of WSP of *Wolbachia* endosymbiont was retrieved from the protein sequence databases, the unknown structure model of WSP was modelled based on homology modelling strategy. The modelled structures were further validated and models were refined using energy minimization technique. At last, the best-constructed model was subjected to the protein-protein interaction study using Fast Fourier Transform (FFT) method called ZDOCK. Further, the clusters and poses were sorted based on the energy value and again fine refining process was introduced through rDock and the best pose protein-protein interaction was reported to prove its binding and Insilico mechanism.

Materials and Methods

Sequence retrieval and search

The sequences of Surface protein from *Wolbachia* endosymbiont of *Exorista sorbillans* was retrieved from UniProt database (http://www.uniprot.org) with unique ID: G1EHU4 in the FASTA format. The known sequence of the unknown structure was given as input for BLASTp by optioning search against Protein Data Bank (PDB) database as a source to identify the similar structure of proteins (https://www.rcsb.org/).

Homology modelling

Homology modelling technique was used to model the structure of *Wolbachia* Surface Protein (WSP). The three-dimensional modelling of WSP was performed according to the template-based modelling strategy that is using known template structure from the structural database. The hit of templates was screened for sequence identity and similarity using align sequence to template tool. The aligned sequence was subjected to homology modelling using build homology model protocol in Discovery studio. This protocol was integrated with MODELLER algorithm to build different WSP structures in automated mode (https://salilab.org/modeller/).

Minimization and Structure validation

In order to select the best model, energy of the modelled protein was computed using calculate energy Tool. In case of structural disorder or unstable energy, the energy minimization technique of various cycles with steepest descent algorithm (SD) or conjugate gradient was applied. In this case, we programmed the 200 cycles using steepest descent algorithm (SD).

Further, we used the Protein Structure Analysis (ProSA) program to check the fitness of the sequences relative to the obtained structures and to assign a scoring function [8] and PROCHECK program to evaluate the stereochemical quality [9]. Also, ERRAT program was used to analyze the statistics of non-bonded interactions between different atom types and to plot the value of the error function versus position of a 9-residue sliding window, calculated by a comparison with statistics from highly refined structures (services.mbi.ucla.edu/ERRAT/).

Protein-protein docking

The fine refined model of WSP was docked with various proteins involved in HepG2 Ethanol stressed pathway. Protein-protein interaction (PPI) were performed using ZDOCK docking algorithm based on the Fast Fourier Transform (FFT) with an angular step of 6 without blocking and specific residues in receptor and ligand.

A clustering poses was optioned to 2000 with maximum ligand interface of 10 Å root-mean-square deviation (RMSD). Also, interface cut off was introduced to increase the number of clusters which reduces the average number of poses per cluster. The top 50 poses were refined further using rDock, consequently the best score results were interpreted for interaction. The list of proteins involved in Ethanol-induced stress pathway of HepG2 cell line is listed in Table 1.

Table 1: Proteins involved in Ethanol-induced stress pathway of HepG2 cell line

List of proteins	PDB ID	UniProt ID
Caspase 3	2C2O	P42574
Bcl-2	4IEH	P10415
Bcl-2-associated X protein	1F16	Q07812
Fas ligand	4MSV	O95407
Fas-associated protein with death domain	1E41	Q13158
Mitogen activated protein kinase	3W8Q	Q02750
c-Jun N-terminal kinases	1JNK	P53779

Results and Discussion

Comparative protein modelling and validation

The template-based modelling of the WSP was performed using MODELLER algorithm. Table 2 illustrates the name of the protein, alignment length, identity and sequence length of the different templates for the query sequence. During Blast against PDB database, it was observed that five templates showed considerable similarity and identity with the query sequence. Since the query hits more than one template so the modeling was carried out using two different methods such as individual template modelling and multiple template modelling.

Table 2: Blast results of templates for query

Name of the protein with Chain	PDB Accession	Identity	Sequence length	Alignment length	E-value	Positives
Chain B, Stonustoxin Structure	4WVM_B	30	700	88	0.25921	47
Chain A, Hemagglutinin-esterase-fusion Mutant Structure of Influenza D Virus	5E5W_A	25	427	108	3.30073	38
Chain A, Crystal Structure of Neisserial Surface Protein A (Nspa)	1P4T_A	27	155	111	5.89423	39
Chain A, Hemagglutinin-esterase-fusion Protein Structure of Influenza D Virus	5E64_A	24	427	108	6.41821	38
Chain A, Nmr Solution Structure of Opa60 from *N. gonorrhoeae*	2MLH_A	57	238	19	6.91165	78

On one hand, multiple template based modelling with all five different protein structures 4WVM_B, 5E5W_A, 5E64_A, 2MLH_A and 1P4T_A did not show significance with sequence to structure alignment. Moreover, it was observed

that the quality of the protein and the arrangement of secondary structure was not appreciable. In addition, structure evaluation on PROCHECK (Ramachandran plot) showed that more than five amino acids are in the disallowed region with poor ProSA and ERRAT quality.

On the other hand, an individual comparative modelling of each template was employed for alignment followed by building protein structure using build homology model. During modelling of structure, the templates like 4WVM_B and 2MLH_A showed discrete alignment with the query sequence, further modelling with that template resulted in poor quality of the model. Whereas, templates 5E5W_A and 5E64_A were good in alignment but the quality of the structure was insignificant. Finally, the modelling with template 1P4T_A a Neisserial Surface Protein A showed good sequence alignment and least positives. Furthermore, the structures built using this template using build homology model protocol depicted good geometry, no bad clashes with other amino acids and less side chain issues. Hence, the structure generated using this template was taken for further proceedings.

Evaluation of structure quality

The modelled structures were validated to know its structure quality. The structure modelled using MODELLER algorithm with template 1P4T (-11,757.60) was selected based on the Discrete Optimized Protein Energy (DOPE) score (Table 3) that is a lower DOPE score which is a statistically better model was selected. The root-mean-square deviation (RMSD) of the structure with its own template (1P4T) was 0.611Å. In addition, structure optimization was done using energy minimization tools, the objective of this process was to reduce the positive energy of the individual amino acid and to increase the stability of the overall structure. Before minimization with steepest descent algorithm, Ramachandran plot showed >4 amino acids in disallowed region. But after minimization results, 0% amino acids were in the disallowed region. Overall, the Ramachandran plot for the optimized structure depicts 86.8% in the favoured region, 11.8% in the allowed regions, 2.6% in the generously allowed regions and 0.0% in the disallowed region (Figure 1). Finally, the modelled structure and template were evaluated in ProSA server. The ProSA server results were found to be satisfactory with values of -3.05 and -2.8 for the template and the modelled structure respectively (Figure 2). Hence the refined structure was taken for protein-protein docking studies.

Table 3: Structure modelled using MODELLER algorithm

Models	DOPE score	RMSD
wsp.M0001	-11,561.90	1.666
wsp.M0005	-11,530.89	0.771
wsp.M0002	-11,510.20	1.262
wsp.M0004	-11,757.60	0.611
wsp.M0003	-11,696.10	0.662

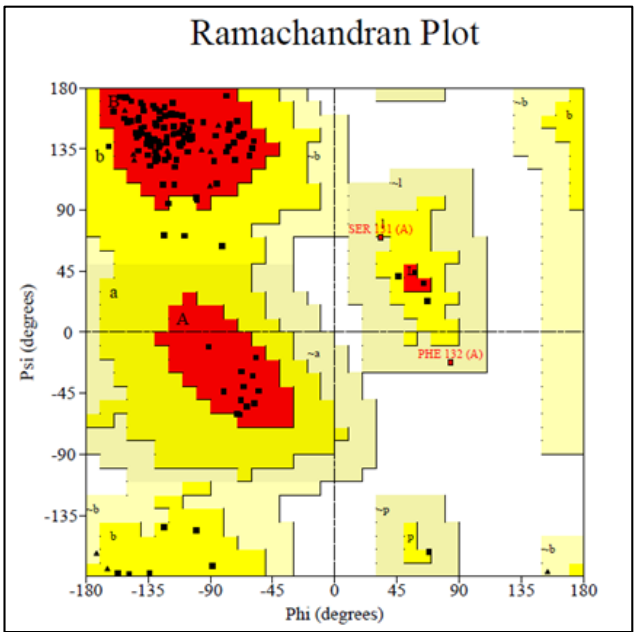

Fig 1: Ramachandran Plot for the best structure

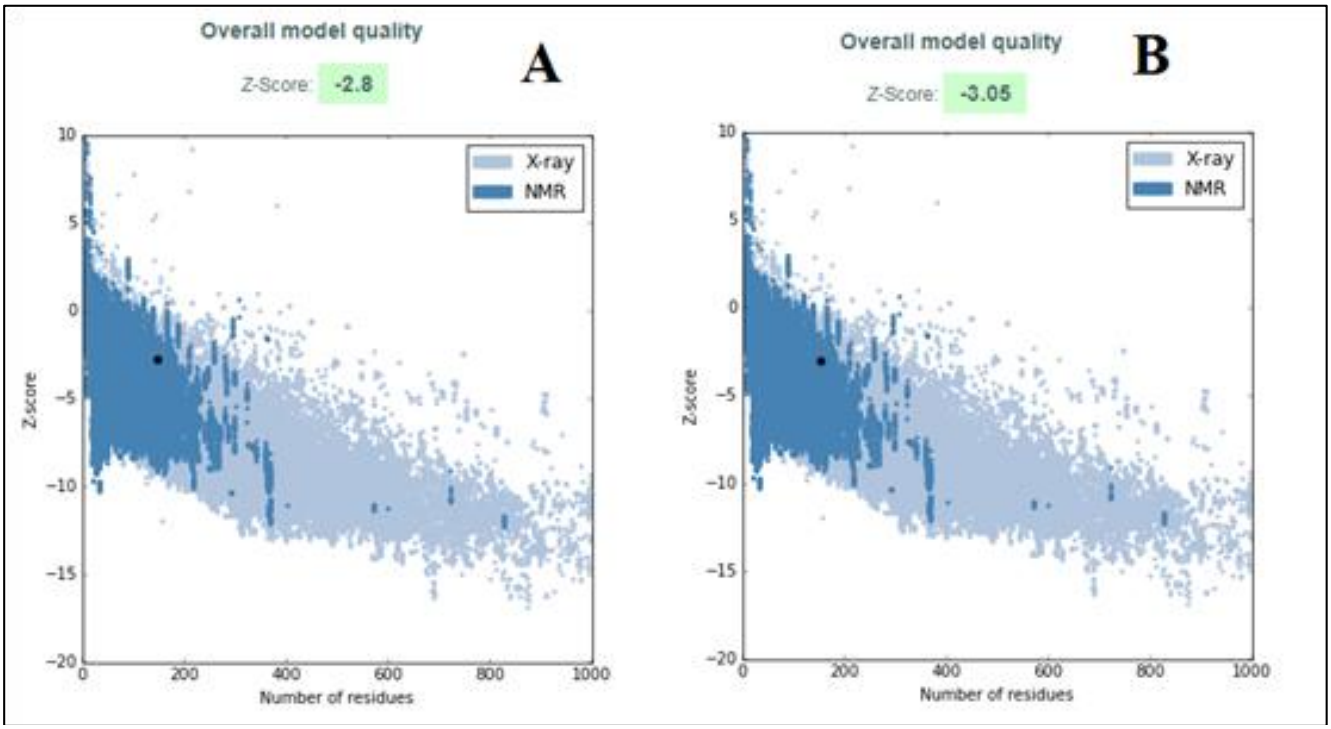

Fig 2: ProSA evaluation A) Modelled structure, B) Template (1P4T)

Comparing architecture of the template with modelled-WSP

As like template structure of Neisserial Surface Protein A (NspA), WSP from Uzifly also formed an eight-stranded antiparallel beta-barrel (Figure 3). The four loops at the extracellular side of the NspA molecule form a long cleft, which contains mainly hydrophobic residues. A β-turn is a type of non-regular secondary structure in proteins that cause a change in direction of the polypeptide chain. Beta turns (β turns, β-turns, β-bends, tight turns, reverse turns) also called Venkatachalam-turns are very common motifs in proteins and polypeptides [10].

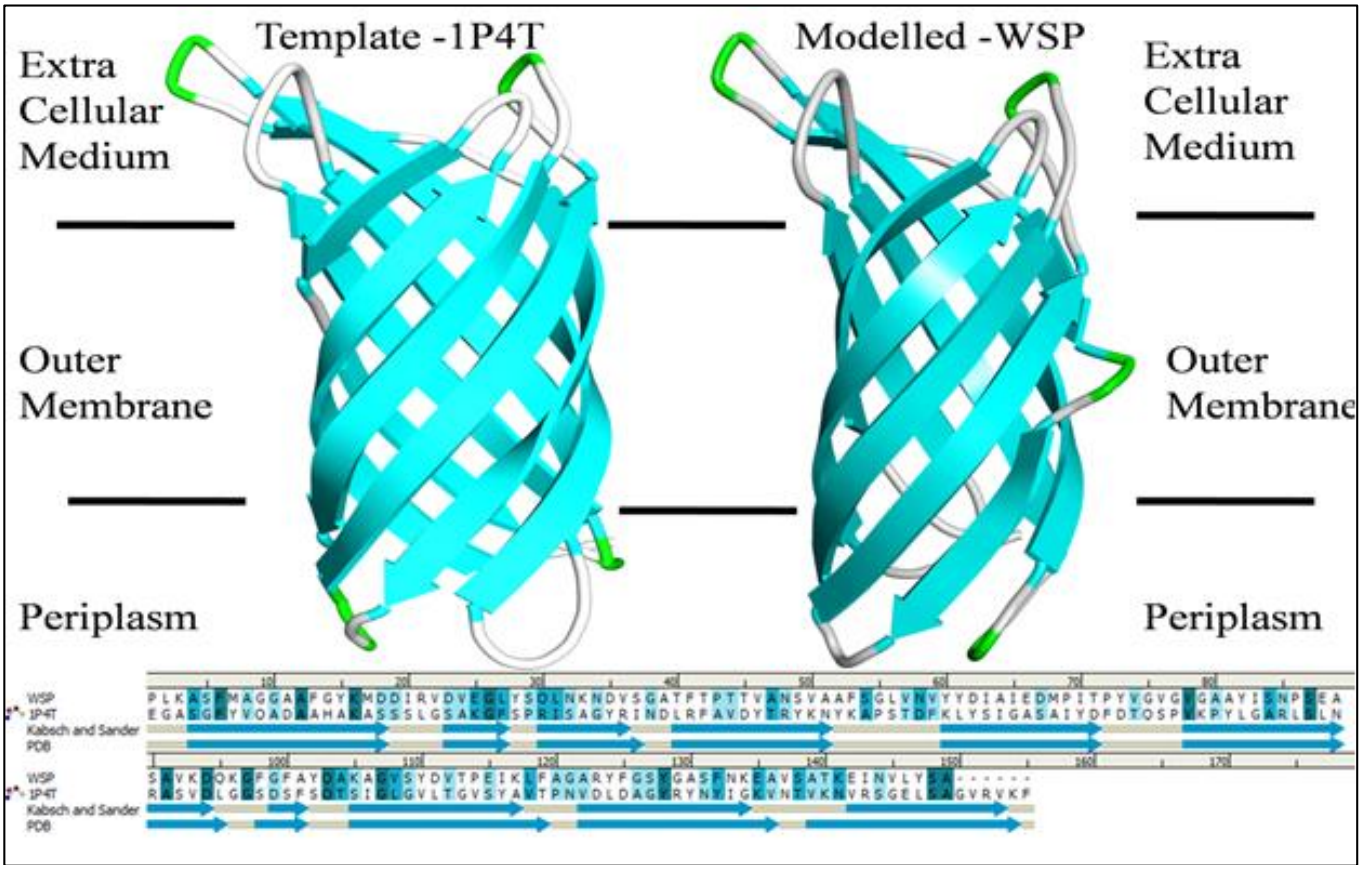

Fig 3: Structural comparison of the template and Modelled WSP structure of Uzifly

Protein-Protein Docking

The proteins involved in the Ethanol stressed pathway of HepG2 cells were retrieved from PDB database, each protein structure was prepared to remove hetatoms, identical chains, water and conformations, these proteins were considered as a receptor and the modelled protein as a ligand. In order to understand the anti-apoptosis process of how the Ethanol stressed HepG2 cell line proteins will interact with WSP, the Docking studies was carried out for selected apoptosis protein with modelled WSP using ZDOCK which resulted in 54000 poses with allowed conformations. However, only 1000 top scoring poses form each protein - WSP complex was retrieved and further sorted based on a large cluster with top poses. The largest cluster contains 12 poses, 15 poses, 13 poses, 17 poses, 21 poses, 14 poses and 15 poses for Caspase 3, Bcl-2, Bcl-2-associated X protein, Fas ligand, Fas-associated protein with death domain, Mitogen-activated Protein Kinase and c-Jun N-terminal kinases respectively.

Moreover, ZDOCK scores were in different range for each protein, these scores were based on the pharmacological effect and the interaction of protein with WSP. For caspase-3 scores were in the range of 17.76 to 11.96, Bcl-2 and Bcl-2-associated X protein were in the range of 19.52 to 11.1 and 19.12 to 12.24 respectively. Similarly, Fas ligand (FasL), Fas-associated protein, Mitogen-activated Protein Kinase and c-Jun N-terminal kinases with death domain scores were in the range of 17.12 to 11.18, 19.54 to 11.36, 19.4 to 12.3 and 21.34 to 12.54 respectively.

The graphical representation of top 50 poses of each protein-protein complex (Protein-WSP) is depicted in Figure 4. Hence, in order to refine the top 50 poses, Refined docking (R dock) was carried out to optimize the docked protein poses utilizing Chemistry at Harvard Macromolecular Mechanics (CHARMM) energy. Finally, the best E_RDOCK scored complex was taken for interface interaction analysis. Table 4 is tabulated with best dock score of all the seven different proteins with WSP. Among various proteins docked with WSP, FasL-WSP protein complex showed least energy (34.0318 kcal/mol) and best docked complex (Figure 5). Another protein c-Jun N-terminal kinases also favoured more to interact with WSP with energy of -33.16 kcal/mol but it ranked second after FasL. Hence interaction analysis was done only for the FasL-WSP protein complex. Thus, from the interaction (Table 5) one can observe the various types of bonding interaction between the two different proteins.

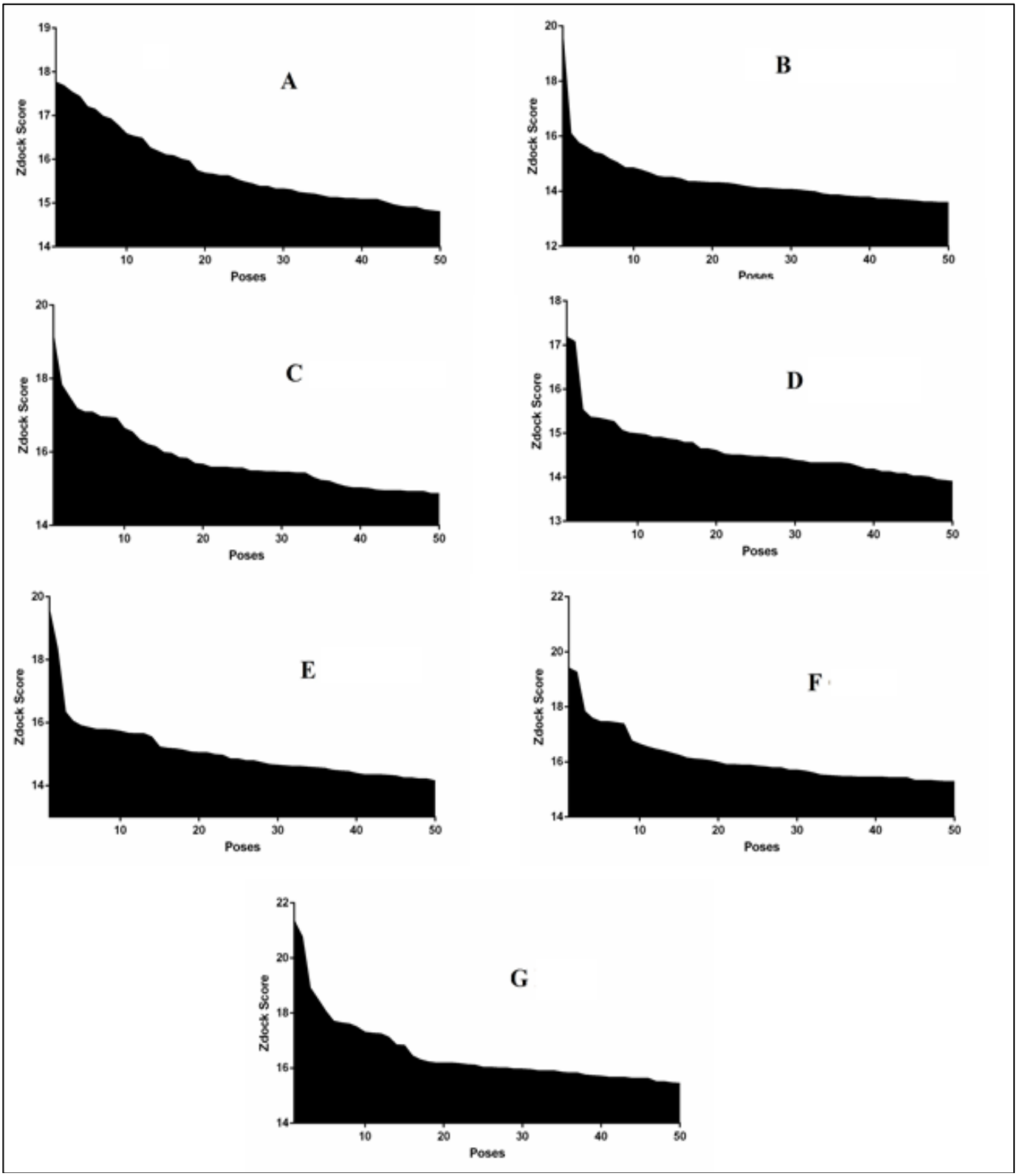

Fig 4: Graphical representation of top 50 poses of Protein-WSP complex
A: Caspase 3 with WSP, **B:** Bcl-2 with WSP, **C:** Bcl-2-associated X protein with WSP, **D:** Fas ligand with WSP, **E:** Fas-associated protein with death domain with WSP, **F:** Mitogen activated protein kinase with WSP, **G:** c-Jun N-terminal kinases with WSP

Table 4: The best dock score of all the seven different proteins with WSP

Proteins	Top Pose	Z-Dock score	E_vdw1	E_elec1	E_vdw2	E_elec2	E_sol	E_RDock
Caspase 3	Pose13	16.26	-98.288	-1.7015	-117.7	-34.079	-8.9	-30.671
Bcl-2	Pose37	13.82	-66.802	-0.7074	-76.507	-15.947	5	-9.3529
Bcl-2-associated X protein	Pose50	14.86	-105.88	-0.7442	-108.01	-9.3377	-12.8	-21.204
Fas ligand	Pose29	14.42	72.7957	-2.1536	-89.336	-5.9242	-28.7	-34.0318
Fas-associated protein with death domain	Pose32	14.6	450.889	-2.3068	5.51101	-14.274	-10	-22.846
Mitogen activated protein kinase	Pose36	15.46	-85.565	-1.8812	-111.25	-23.039	0.2	-20.535
c-Jun N-terminal kinases	Pose26	16.02	-45.714	-2.7016	-101.86	-6.7332	-27.1	-33.16

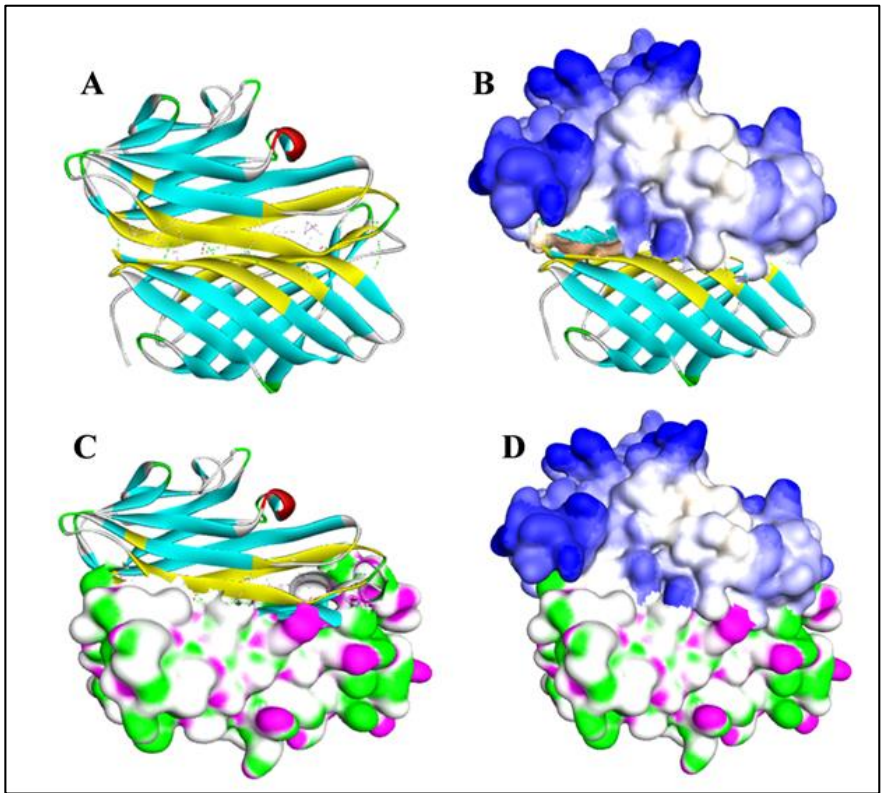

Fig 5: The best pose 29 structures in different representations for Fas L-WSP protein complex
A: Secondary structure of receptor (Fas L) and ligand (WSP), **B:** Hydrophobicity Surface of receptor (Fas L) and secondary structure of ligand (WSP), **C:** Hydrogen Bond Donor/Acceptor Surface (WSP) and Hydrophobicity Surface (Fas L) **D:** Hydrogen Bond Donor/Acceptor Surface (WSP) and secondary structure of receptor (Fas L)

Table 5: Interaction analysis of Fas L - WSP complex

Protein-Protein Interaction	Distance	Types of Bond
A:GLU226:HN - :GLY10:O	2.38659	Conventional Hydrogen Bond
A:LYS228:HN - :ALA12:O	2.84079	Conventional Hydrogen Bond
A:LYS228:HN - :ILE143:O	2.23684	Conventional Hydrogen Bond
A:THR234:HG1 - :GLU25:OE1	2.16447	Conventional Hydrogen Bond
A:ARG241:HH11 - :VAL46:O	1.91265	Conventional Hydrogen Bond
A:ARG241:HH22 - :ASN48:OD1	1.98825	Conventional Hydrogen Bond
A:ASN250:HN - :ALA149:OCT2	2.05675	Conventional Hydrogen Bond
:SER29:HG - A:TYR244:O	3.03536	Conventional Hydrogen Bond
:ASN48:HD21 - A:THR234:O	2.8817	Conventional Hydrogen Bond
:SER49:HG - A:THR234:OG1	2.97502	Conventional Hydrogen Bond
:VAL145:HN - A:GLU226:O	2.45144	Conventional Hydrogen Bond
:TYR147:HN - A:MET224:O	3.00414	Conventional Hydrogen Bond
A:ARG241:CD - :LEU27:O	3.51236	Carbon Hydrogen Bond
A:SER243:CA - :SER29:OG	3.2684	Carbon Hydrogen Bond
:GLY14:CA - A:MET230:O	2.90366	Carbon Hydrogen Bond
:SER29:CB - A:SER242:O	3.47326	Carbon Hydrogen Bond
:LYS141:CE - A:MET230:O	3.04865	Carbon Hydrogen Bond
:TYR147:CD2 - A:PHE249	3.82308	Pi-Sigma
A:LEU222 - :ILE116	5.33469	Alkyl
A:VAL223 - :ILE116	5.40956	Alkyl
A:VAL223 - :VAL145	4.1224	Alkyl
A:LYS228 - :ILE143	4.29274	Alkyl
A:MET229 - :LEU27	3.76296	Alkyl
A:ARG241 - :LEU27	3.62514	Alkyl
A:ARG241 - :VAL46	4.52605	Alkyl
A:ALA247 - :ALA8	3.42026	Alkyl
A:ALA247 - :LYS33	4.24652	Alkyl
:ALA8 - A:MET225	4.50643	Alkyl
:ALA12 - A:MET229	3.39571	Alkyl
A:TYR232 - :LEU27	4.65602	Pi-Alkyl
A:TYR244 - :LEU31	5.02317	Pi-Alkyl
A:PHE249 - :ALA8	4.07392	Pi-Alkyl
:TYR147 - A:LEU222	4.72875	Pi-Alkyl
:TYR147 - A:MET224	4.86873	Pi-Alkyl

For Fas L-WSP protein complex, different types of bonding interaction was observed which includes Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Sigma, Alkyl and Pi-Alkyl. Previously, the anti-apoptosis process in HepG2 cell line has been reported widely on compounds like Phyllanthus [11], but in this study one can understand the cytoprotective process of WSP atomic level interaction with the proteins expressed in ethanol/alcohol stressed HepG2 cell line.

Conclusion

Therefore, Anti-apoptosis in cell is a vital process to protect the cell from death. Studies for cytoprotective drugs have been reported previously. The novelty of this study was WSP from Uzifly was used and studied its pharmacological action using bioinformatics application. Consequently, the binding energy levels of WSP with various proteins showed the antagonism. Among seven proteins docked with WSP, Fas L-WSP complex showed the most prominent way of reducing the apoptosis process thereby protecting the cell from death. Thus, WSP may be used as drug candidate for the treatment of Ethanol-related liver diseases.

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